PROCEEDINGS OF THE CALIFORNIA ACADEMY OF SCIENCES

Volume 56, No. 16, pp. 170–178, 6 figs. April 29, 2005

Structural, Biochemical and Molecular Investigations on Wadden Sea Diatoms: Field Studies and Laboratory Experiments

Telse Meyer[2], Julia Sauer[2], Andreas Oeltjen[2], Klaus Wenderoth[1], Jürgen Marquardt[2], Erhard Rhiel[2,3]

[1] *Fachbereich Biologie, Botanik, AG Zellbiologie, Philipps-Universität Marburg, Karl-von-Frisch-Strasse, Lahnberge, D-35032 Marburg/Lahn, Germany;*

[2] *Geomikrobiologie, ICBM, Carl-von-Ossietzky-Universität Oldenburg, P.O.B. 2503, D-26129 Oldenburg, Germany; Tel: 0441-798-3389; Fax: 0441-798-3384*

Wadden Sea-inhabiting diatoms

Wadden Sea sediments are inhabited mainly by diatoms, euglenoids and cyanobacteria. Diatoms may dominate these habitats with respect to species diversity and cell density and often exceed cell numbers of more than 10^6 per cm^2 sediment. Several sediment-inhabiting diatom species show a rhythmic vertical migration (VM) behaviour and move up onto the surface when the water drains off and down into the sediment bed with the incoming tide (Harper 1969, 1976; Happey-Wood and Jones 1988; Hopkins 1966; Palmer and Round 1965; Round and Happey 1965; Round and Eaton 1966). They, thus, have to face dramatic changes of several physical and chemical parameters in their natural habitat: tidal inundation results in reduced concentrations of gases and lowered light intensities, but gives rise to almost constant values of temperature, salinity, pH and nutrients. During tidal emersion these parameters may vary drastically: salinity and temperature may increase and lead to desiccation and temperature stresses, respectively, whereas light intensities of up to 2000 µmol photons m^{-2} s^{-1} may cause photoinhibition (Underwood 2002). The dramatic changes will have a great impact on the community structure on a long-term scale (months and years) and, on a short time scale (minutes and hours), will result in adaptation processes within the cells. For investigating both kind of responses, temporal changes in community structure and short-term cellular adaptation processes, it is necessary to use "old-fashioned" classical methods of diatom research and "modern," i.e., molecular, biophysical, and biochemical methods in parallel. In the following sections, we give a short summary of the methods used in our lab to study Wadden Sea diatoms. The methodological approaches have now been well established for laboratory experiments, and it is a challenge to transfer and use this repertoire of methods directly in the field or on field samples.

Classical Taxonomy and Identification of Wadden Sea-inhabiting Diatoms

To study long-term changes of the diatom community the use of classical methods for diatom identification are indispensable. Usually, acid-cleaned diatom frustules are used for this purpose. Wadden Sea sediment samples collected at regular intervals over periods of several months or even

[3] Corresponding author. Email: *erhard.rhiel@uni-oldenburg.de.*

years from the same sampling area can be easily treated with HCl and H_2O_2 as oxidative agents followed each by repetitive washing steps with water. Light microscopy and scanning electron microscopy give information on species diversity and species abundances. These data allow statements on local and temporal successions of distinct species at a given habitat. A major disadvantage of this approach is that no information on the distribution of individual cells within the sediment bed becomes available. For this, sediment cores have to be withdrawn, frozen and sectioned horizontally into slices of 200 to 500 µm width (de Brouwer and Stal 2001; Kelly et al. 2001). The slices can also be used for frustule preparations.

Laboratory Tidal Micro-ecosystem and Mini-mesocosms

Vertical movement can be investigated in the laboratory under almost natural conditions. Sediment samples collected in the field and transferred to the laboratory do not show fluctuations in species abundance during the first two weeks after transfer (Defew et al. 2002). During this time period, tidal immersion and tidal emersion can be simulated in tidal micro-ecosystems (Paterson 1986). Alternatively, sediment samples can be put into mini-mesocosms, i.e., aluminium stubs (Fig. 1) or small petri dishes, which are placed in wet chambers and subjected to defined regimes of several physical and chemical parameters: light intensity, light quality, temperature, pH, salinity, or gas availability may be varied each separately while leaving the others constant (see Fig. 2). After incubation, the cell densities on the sediment surfaces are evaluated by means of epifluorescence light microscopy or low-temperature scanning electron micros-copy (see below). Thus, data on the effects of these external parameters on the VM and species abundances become available.

Figure 1: Mini-mesocosms in aluminium stubs of 4 mm height and 10 mm diameter. The cavities are 3 mm in depth and 6 mm in diameter (A). Small aliquots of thoroughly homogenized sediment samples are transferred into the cavities of aluminium stubs (B). These can be placed in humid chambers under defined conditions of e.g. light, temperatur and salinity. After cryofixation, the stubs are mounted onto a specimen holder (C) and transferred into cryochamber attached to a scanning electron microscope.

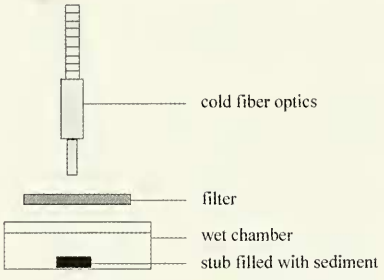

Figure 2: Experimental setup for investigating the effects of external factors on the VM. Small petri dishes or aluminium stubs filled with sediments are placed in a humid chamber. Light is supplied from above by using cold fiber optics. Gray or interference filters may be used to alter either light intensity or light quality. The temperature may varied by placing the wet chambers onto a cooling/heating device. Furthermore, the chambers can be flushed with gases via inlet/outlet ports (not shown in the figure).

Low-temperature Scanning Electron Microscopy (LTSEM) and Epifluorescence Light Microscopy

LTSEM and epifluorescence light microscopy of aluminium stubs or small petri dishes filled with sediment allow the investigation of diatom migration and lead to similar results. LTSEM was first introduced for the investigation of Wadden Sea sediments by Paterson (1986) and allows the observation of sediments and diatoms living therein in an almost natural state. The fixation procedure of sediment samples during cryofixation was further improved by the development of the Cryolander device (Whiltshire et al. 1997). Using LTSEM, data on diversity and abundances of species covering the sediment surface be-come available (see Fig. 3A). If sampling is performed at regular intervals during a complete tidal cycle, it is even possible to deter-

FIGURE 3: (A) Electron micrograph of a sediment surface visualized by LTSEM. Diatom cells dominate the surface. Beside, a filamenteous cyanobacterium can be seen. (B) LTSEM electron micrograph of a sediment sample which has been freeze-fractured perpendicularly to the sediment surface. The location and distribution of cells within the sediment bed become visible.

mine at which time distinct species show VM (Paterson 1986). The latter can also be done by epifluorescence light microscopy or by using the lens tissue technique of Eaton and Moss (1966). LTSEM, however, allows the collection of this information on natural sediments, which have been cryofixed in the field, or from those kept in mini-mesocosms (Janssen et al. 1999; Sauer et al. 2002). When sediment samples become freeze-fractured perpendicularly to the sediment surface, LTSEM even allows investigation of the location and distribution of cells within the sediment bed (see Fig. 3B).

Sauer et al. (2002) found that the migration of diatoms onto the sediment surface was partially inhibited by darkness but strongly enhanced by light. Inhibition and enhancement were reversible. These results are in accordance with data of Palmer and Round (1967) for *Hantzschia* sp. Increasing light intensities (up to 500 µmol photons m^{-2} s^{-1} were applied) led to rise in cell numbers on the sediment surface and resulted in variations in the composition of species migrating to the sediment surface; large species like *Gyrosigma* surfaced under low fluence rates whereas naviculoid species surfaced under higher fluence rates (Sauer et al. 2002). Changes in the composition of surfacing species have already been described by Round and Palmer (1966) and Paterson (1986). Sauer et al. (2002) found that the upward migration in the morning was most responsive to light. When the sediment samples were remixed or mixed around noon or in the afternoon, fewer cells migrated to the surface. Thus, it might be that the sensitivity to the light stimulus is highest in the early morning hours or immediately after tidal cover had drained off. A maximum upward migratory behaviour was found at 35 ppm salinity. Fewer cells surfaced when the salinity was either lowered or increased. Higher salinities might occur during hot and/or windy days and nights after prolonged exposure of the sediment surfaces whereas lower salinity values might be caused by rainfall or in inlets of estuarine waters.

Migratory speed is assumed to be an important factor responsible for the accumulation of the diatoms on the sediment surface. The migratory speed averaged over the entire diatom population was approximately 1 µm/s and remained similar during experiments in which salinity was either lowered to 5 ppm or increased to 60 ppm (Sauer et al. 2002). This value is rather low and does not necessarily reflect the migratory speed that diatoms will show when moving in natural substrates as the horizontal migration speed was measured in cuvettes. Hay et al. (1993) reported an average speed of 4.7 µm/sec for a *Gyrosigma* species over an artificial sediment surface (kaolin) but a much slower migration speed of 0.17 to 0.19 µm/sec. when the diatom moved through a sediment bed.

The light quality also has a great impact on the upward directed VM. Thus, diatoms surface most when illuminated with blue light; almost no response was registered under green light, whereas illumination with red light seems to have some positive effect on the migration onto the sedi-

ment surface (Wenderoth and Rhiel 2004). Several classes of photoreceptors with defined functions and absorbance characteristics have been described for plants, and the results indicate that most probably the cryptochromes, which absorb blue light, and the phytochromes, which absorb in the red light/far red light region, are involved.

Time-lapse Video Microscopy

Time-lapse video microscopy is another excellent tool to study the migration behaviour, i.e., migratory speed and migration on/in natural/artificial sediments or horizontal cuvettes. Individual cells or cell assemblages can be tracked for hours and days. Although LTSEM finally results in cryofixation and death of living cells, time-lapse video microscopy allows investigations without damaging them. Single pictures and videos can be recorded using framegrabber software and hardware and stored as digitized files (pictures as TIF or JPG formatted files, video clips as MPG or AVI files) on a personal computer (Sauer et al. 2002). An example is given in Figure 4. It shows that the trails of individual large cells, most probably species of the genera *Pleurosigma* or *Gyrosigma*, were used by other, smaller diatoms, most probably *Navicula* species, for upward directed VM (Wenderoth et al. 2004). The movement of three large diatoms was monitored. One cell moved up and down the cuvette on the same trail, from the upper to the lower edge of the screen before it disappeared at the lower left corner. Its trail was used by smaller cells for upward migration. The other larger cell moved from the upper to the right edge where it disappeared. Later, a third large cell, which may or may not be the same individual as the second one, moved in from the right edge and formed a new trail moving downwards and upwards before it disappeared in the lower right corner. It was followed by smaller cells, which soon afterwards used all the trails formed by the large cells for upward VM.

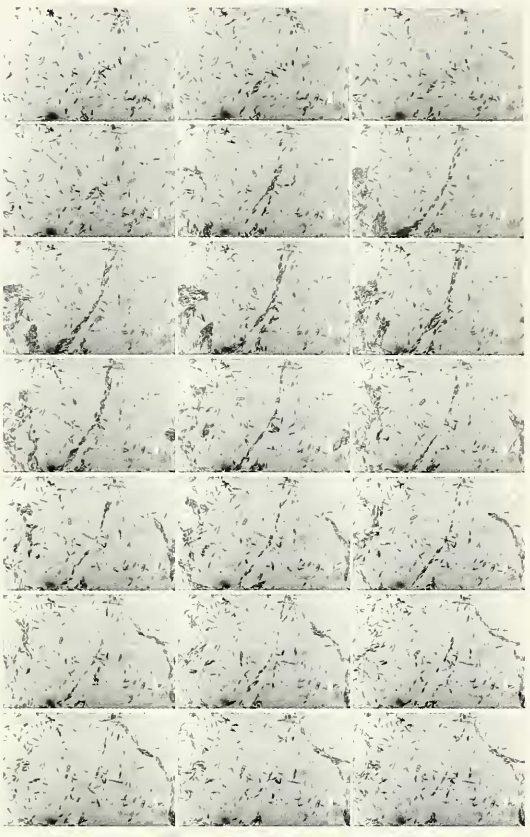

Figure 4: Images taken by time-lapse video microscopy showing the movement of diatoms in the vertical cuvette. For the video documentation, a small amount of sediment was placed onto the bottom of a home made cuvette which was positioned vertically in front of a horizontally fixed light microscope. The cuvette was floated with artificial seawater and illuminated from above. Near infrared light for video microscopy was provided by a projector using a 740 nm interference filter. The migratory behaviour of diatoms was documented immediately above the sediment sample in the cuvette over a time period of 24 hours using a 12.5× Leitz achromatic objective in combination with a Panasonic B/W CCD camera and a time lapse video cassette recorder. The video tape was digitized on a personal computer to an AVI file. Representative single pictures taken from the video clip are shown with the first one in the upper left and the last one in the lower right corner of the figure. The area size of the pictures is 700 µm × 500 µm.

Nucleic Acid and Protein Work

Endogeneous rhythms in gene expression as well as short-term adaptation processes on external factors such as fluctuating light intensities will result in fluctuating mRNA and polypeptide abundancies. The polypeptides, constituting the light-harvesting (LH) complexes, are an excellent starting material for studying gene expression and protein quantities of Wadden Sea-inhabiting diatoms. Both the genes encoding LH polypeptides (*lhc* genes) and the LH polypeptides and their mRNAs are highly abundant and have already been investigated in detail in higher plants and green algae: lhc genes exhibit an endogenous circadian rhythmic expression pattern with increasing amounts of mRNA from the onset of light towards noon and decreasing amounts in the afternoon towards night. This expression pattern results in almost concomitant enhanced amounts of newly synthesized LH polypeptides in the morning and reduced synthesis in the afternoon. *lhc* mRNA and LH polypeptide abundancies are further regulated by external factors such as light (intensity and quality), temperature, and nutrient availability. In diatoms, the counterparts to the *lhc* genes and LH polypeptides of higher plants and green algae are the *fcp* genes and their corresponding gene products, the fucoxanthin chlorophyll *a/c* binding polypeptides (*Fcp*). Data on *fcp* gene expression and *Fcp* abundancies are rare and almost restricted to laboratory studies. Eight *fcp* genes of the centric diatom, *Cyclotella cryptica*, have been cloned from a cDNA library and investigated in detail (Eppard and Rhiel 1998; Eppard and Rhiel 2000; Eppard et al. 2000). Phylogenetic analyses show that they can be grouped into three distinct clusters. Cluster I harbours the *fcp* genes *fcp1, fcp2, fcp3* and *fcp5*. They are similar to the *fcp* genes known from other diatoms and brown algae. Cluster II contains the *fcp* genes *fcp6, fcp7* and *fcp12*, which are more closely related to light-inducible *lhc*-related genes, which have been cloned from the green alga, *Chlamydomonas*. In cluster III, currently one *fcp* gene, *fcp4*, has been placed which shows the highest homology to the *lhc* genes cloned from the red alga, *Porphyridium cruentum*. The transcript sizes of fcp genes of *Cyclotella cryptica*, belonging to different *fcp* gene clusters and encoding different *Fcps*, the diurnal expression of the *fcp1/fcp2/fcp3/fcp5* gene cluster and the steady-state mRNA concentrations of all gene clusters in response to light quality and quantity were investigated by Oeltjen et al. (2002). The mRNAs of the gene cluster I are approximately 950 bases in length, whereas those of the *fcp4* and the fcp6/fcp7/fcp12 clusters are approximately 1050 (*fcp4*), 880 (*fcp6/fcp7*) and 1150 (*fcp12*) bases in lengths. Similar to what is found for higher plants, the steady-state mRNA concentration of the *fcp1/fcp2/fcp3/fcp5* gene cluster increased with the onset of light, reached a maximum around noon and dropped in the afternoon. The steady-state mRNA concentrations of the genes belonging to cluster I and of the *fcp4* gene were higher when *C. cryptica* was grown in low light, whereas the steady-state mRNA concentration of the *fcp6/fcp7/fcp12* gene cluster increased under high light growth conditions. The steady-state mRNA concentrations of all gene clusters were highest when *C. cryptica* was grown in red light, intermediate in green light, and lowest in blue light. The *fcp* genes, especially those belonging to the gene cluster I, and a polyclonal antiserum raised against the *Fcps* of *C. cryptica* are excellent tools for studying steady-state fcp mRNA concentrations and *Fcp* abundances of diatoms directly in the field. The methodological approaches were published by Hust et al. (1999) and Meyer et al. (2003); they are summarized in the flow chart of Figure 5.

Generally, the results using these techniques are in line with those obtained in laboratory experiments on unialgal cultures. Hust et al. (1999) found that repetitive extractions with sodium dodecylsulfate (SDS) containing sample loading buffer used for SDS-polyacrylamide gel electrophoresis ensured that more than 98% of the extractable protein was recovered. Subsequent Western immunoblotting with the *Fcp*-antiserum selectively immunodecorated *Fcps* and, thus, demonstrates that a taxon-specific class of polypeptides can be visualized and quantified directly

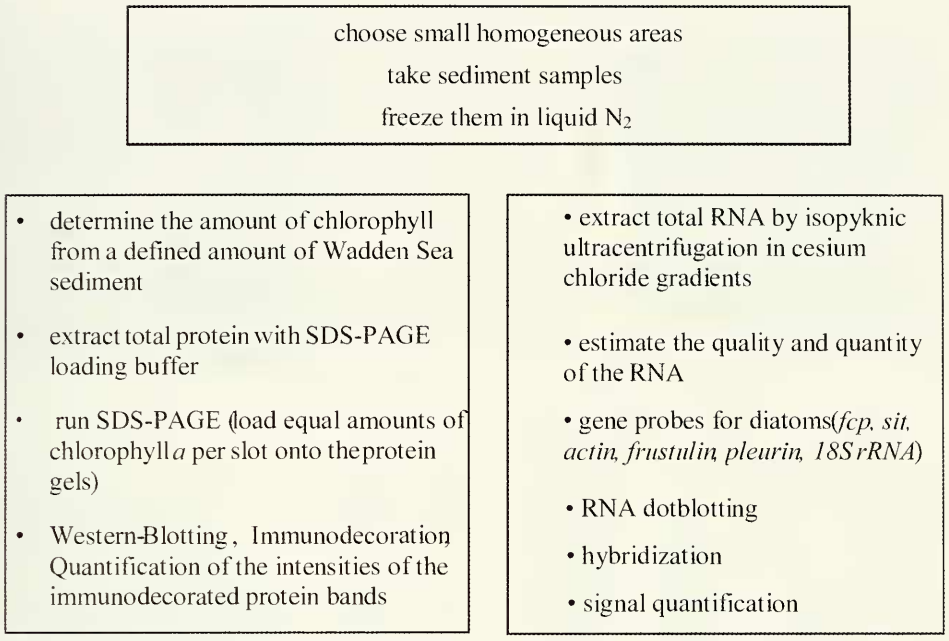

FIGURE 5: Flow chart for the isolation of total protein (left) or total RNA (right) out of Wadden Sea sediments.

in sediment samples. In shading experiments, shaded sediment areas generally revealed higher amounts of *Fcp* subunits which could be immunodecorated.

Meyer et al. (2003) established a method to investigate the steady-state levels of *fcp* mRNAs of diatoms *in situ*. Field samples were taken after tidal exposure from dawn to late afternoon at two-hour intervals and frozen in liquid nitrogen. In the laboratory, total RNA was isolated by isopyknic ultracentrifugation in cesium chloride gradients. Defined amounts of total RNA were blotted onto nylon membranes and hybridized with probes against the *fcp2* and 18S rDNA genes of *C. cryptica*. The steady-state amount of *fcp* mRNAs was estimated by normalizing the *fcp* signal intensities to the signal intensities obtained from hybridization experiments, in which the 18S rDNA gene probe was used (see flow chart, Fig. 5). In time course studies, which were performed to demonstrate the applicability of the method, the steady-state levels of *fcp* mRNA increased up to 12-fold with the onset of light. Similar to what has been found in laboratory experiments on unialgal cultures, the levels reached a maximum 6–8 h after sunrise before they decreased again. Further results using the methodological approaches described above are given in Figure 6. Here, Wadden Sea sediments were withdrawn during the morning hours up to 2 pm in the afternoon and subjected to RNA and protein isolation. For both the *Fcp* abundances and steady-state *fcp* mRNA concentrations, an increase towards the early afternoon could be demonstrated.

FUTURE PROSPECTS

At present, neither the effects of chemical gradients (O_2, CO_2, S^{2-}, NO_3^-) nor the impact of physical gradients such as temperature and pH on the VM have been investigated. The influences of excessive light (> 500 µmol photons m^{-2} s^{-1}) and gravitaxis on the VM have not been elucidated either. Further experiments have to be undertaken to unravel the individual impacts of the entire set of these factors.

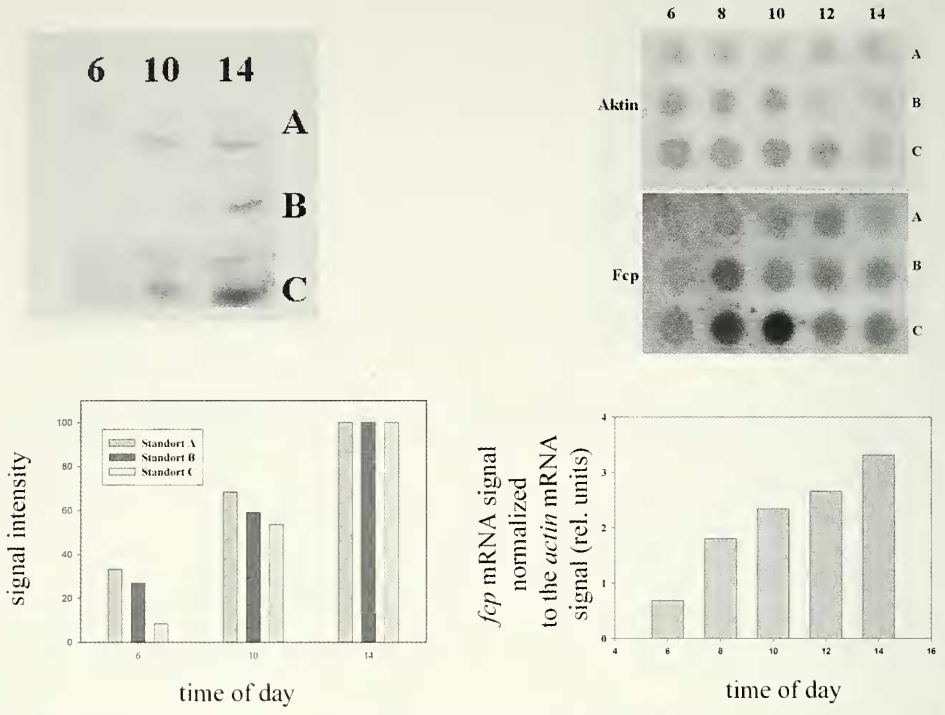

FIGURE 6: Left side, upper part: Western immunoblots of total protein extracted from three sediment sampling sites (A, B, and C). Sampling was performed at 6 am, 10 am and 2 pm, respectively. The proteins were blotted onto nitrocellulose after electrophoresis and immunolabeled with an antiserum directed against the Fcp subunits of the diatom *C. cryptica*.

Left side, lower part: The signal intensities obtained from quantitative analysis of the immunodecorized bands are presented as bars and plotted against the time of day; the highest signal intensity is set to 100 %.

Right side, upper part: Representative dot blots of 10 µg RNA isolated from three Wadden Sea sediment sampling sites (A, B, and C) and hybridized with either the fcp2 gene probe or the actin gene probe. The values above the dots show the local time of sample withdrawal. The gene probes are indicated on the left.

Right side, lower part: Bar graph showing the steady state level of Wadden Sea sediment fcp mRNA, which was estimated by normalizing the fcp signal intensities to the corresponding actin mRNA signal intensities. Abscissa: time of day, ordinate: normalized steady state fcp mRNA levels.

Additional molecular techniques have to be adjusted and applied: total DNA and RNA can be isolated from sediment samples which is suited for polymerase chain reaction (PCR-) mediated amplification of diatom-specific 18S rDNA or mRNA after being reverse-transcribed. The PCR products could be used for standard molecular techniques such as Single Stranded DNA Conformation Polymorphism (SSCP), Denaturing Gradient Gel Electrophoresis (DGGE), or Differential Display. The PCR products can be subjected to routine DNA sequencing protocols to yield information on the species from which they were derived as well as on species diversity. *In-vivo* cell labelling, in combination with confocal light microscopy, will allow tracing of individual species within their natural habitat by using species-specific gene probes derived from the sequencing data and carrying a fluorescence label. Antisera directed against proteins that play major roles in main enzymatic pathways (e.g., uptake of nitrate, phosphate, silicate, synthesis and excretion of polymers) will allow a closer look at protein abundancies, whereas the corresponding gene probes will allow conclusions on gene expression.

Diatoms are fascinating microorganisms. It is no matter of debate that they have attracted the

attention of "observing and describing" scientists first: microscopists and taxonomists. As new methods, i.e., molecular, biochemical, and biophysical, have become available, it is — also no matter of debate — a challenge and chance to use these on diatoms in their natural habitats as well. In the future, modern methods will allow testing of species concepts and will surely yield information on how diatoms live and survive in their natural habitats.

Acknowledgements

The authors express their graditude to Ms. Renate Kort and Mr. Michael Pilzen for technical assistance. They further would like to thank Prof. Chris Bowler (Statione A. Dohrn, Naples, Italy) for kindly providing the actin gene probe. The financial support of the Deutsche Forschungsgemeinschaft (Rh 20/3) is gratefully acknowledged.

References

De Brouwer, J.F.C., and L.J. Stal. 2001. Short-term dynamics in microphytobenthos distribution and associated extracellular carbohydrates in surface sediments of an intertidal mudflat. *Marine Ecology Progress Series* (218):33–44.

Defew, E.C., Paterson, D.M., and S.E. Hagerthey. 2002. The use of natural microphytobenthic assemblages as laboratory model systems. *Marine Ecology Progress Series* (237):15–25.

Eaton, J.W., and B. Moss. 1966. The estimation of numbers and pigment content in epipelic algal populations. *Limnology and Oceanography* 11:584–595.

Eppard, M., W.E. Krumbein, A. von Haeseler, and E. Rhiel. 2000. Characterization of fcp4 and fcp12, two additional genes encoding light-harvesting proteins of *Cyclotella cryptica* (Bacillariophyceae) and phylogenetic analysis of this complex gene family. *Plant Biology* 2: 283–289.

Eppard, M., and E. Rhiel. 1998. The genes that encode light-harvesting subunits of *Cyclotella cryptica* (Bacillariophyceae) constitute a complex and heterogeneous multigene family. *Molecular and General Genetics* 260:335–345.

Eppard, M., and E. Rhiel. 2000. Investigations on gene copy number, introns and chromosomal arrangement of genes encoding the fucoxanthin chlorophyll a/c-binding proteins of the centric diatom *Cyclotella cryptica*. *Protist* 151:27–39.

Happey-Wood, C.M., and P. Jones. 1988. Rhythms of vertical migration and motility in intertidal benthic diatoms with particular reference to *Pleurosigma angulatum*. *Diatom Research* 3:85–95.

Harper, M.A. 1969. Movement and migration of diatoms on sand grains. *British Phycological Journal* 4:97–103.

Harper, M.A. 1976. Migration rhythm of the benthic diatom *Pinnularia viridis* on pond silt. *New Zealand Journal of Marine and Freshwater Research* 10:381–384.

Hay, S.I., T.C. Maitland, and D.M. Paterson. 1993. The speed of diatom migration through natural and artificial substrata. *Diatom Research* 8:371–384.

Hopkins, J.T. 1966. Some light-induced changes in behaviour and cytology of an estuarine mud-flat diatom. Pages 335–358 *in* R.Bainbridge, ed., *Light as an Ecological Factor.* Symp. Br. Ecol. Soc., Blackwell Scientific Publications, Oxford.

Hust, M., W.E. Krumbein, and E. Rhiel. 1999. An immunochemical in situ approach to detect adaptation processes in the photosynthetic apparatus of diatoms of the Wadden Sea sediment surface layers. *Journal of Microbiological Methods* 38:69–80.

Janssen, M., M. Hust, E. Rhiel, and W.E. Krumbein. 1999. Vertical migration behaviour of diatom assemblages of Wadden Sea sediments (Dangast/Germany): A study using Cryo-scanning electron microscopy. *International Microbiology* 2:103–110.

Kelly, J. A., C. Honeywill, and D.M. Paterson. 2001. Microscale analysis of chlorophyll-*a* in cohesive intertidal sediments: the implications of microphytobenthos distribution. *Journal of the Marine Biological Association of the UK* 81:151–162.

Meyer, T., M. Hust, J. Marquardt, W.E. Krumbein, and E.Rhiel. 2003. A methodological approach to investigate the steady state fcp mRNAs levels in Wadden Sea sediments. *International Microbiology* DOI 10.1007/s10123-003-0098-z.

Oeltjen, A., W.E. Krumbein, and E. Rhiel. 2002. Investigations on transcript sizes, steady state mRNA concentrations and diurnal expression of genes encoding fucoxanthin chlorophyll a/c light harvesting polypeptides in the centric diatom *Cyclotella cryptica*. *Plant Biology* 4:250–257.

Palmer, J.D., and F.E. Round. 1965. Persistent, vertical-migration rhythms in benthic microflora 1. The effect of light and temperature on the rhythmic behaviour of *Euglena obtusa*. *Journal of the Marine Biological Association U.K.* 45:567–582.

Palmer, J.D., and F.E. Round. 1967. Persistent, vertical-migration rhythms in benthic microflora VI. the tidal and diurnal nature of the rhythm in the diatom *Hantzschia virgata*. *Biological Bulletin Marine Biological Laboratory Woods Hole* 132:44–55.

Paterson, D.M. 1986. The migratory behaviour of diatom assemblages in a laboratory tidal micro-ecosystem examined by low temperature scanning electron microscopy. *Diatom Research* 1:227–239.

Round, F.E., and J.W. Eaton. 1966. Persistent, vertical-migration rhythms in benthic microflora 3. The rhythm of epipelic algae in a freshwater pond. *Journal of Ecology* 54:609–615.

Round, F.E., and C.M. Happey. 1965. Persistent, vertical-migration rhythms in benthic microflora, Part IV A diurnal rhythm of the epipelic diatom association in non-tidal flowing water. *British Phycological Bulletin* 2:463–471.

Round, F.E., and J.D. Palmer. 1966. Persistent , vertical-migration rhythms in benthic microflora II. Field and laboratory studies on diatoms from the banks of the river Avon. *Journal of the Marine Association. U.K.* 46:191–241.

Sauer, S., K. Wenderoth, U.G. Maier, and E. Rhiel. 2002. Effects of salinity, light and time on the vertical migration of diatom assemblages. *Diatom Research* 17:189–203.

Underwood, G.J.C. 2002. Adaptations of tropical marine phytobenthic assemblages along a gradient of light and nutrient availability in Suva Lagoon, Fiji. *European Journal of Phycology* 37:449–462.

Wenderoth, K, J. Marquardt, and E. Rhiel. 2004. The big trail: many migrate on the expense of a few. *Diatom Research* 19(1):115–122.

Wenderoth, K, and E. Rhiel. 2004. Influence of light quality and gassing on the vertical migration of diatoms inhabiting the Wadden Sea. *Helgoland Marine Research* 58:211–215. (DOI 10.1007/s10152-004-0187-1)

Whiltshire, K.H., J. Blackburn, and D. M. Paterson. 1997. The Cryolander: a new method for fine scale *in situ* sampling of intertidal surface sediments. *Journal Sedimentary Research* 67:977–981.